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GDNF and Addiction

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SYNOPSIS

Biochemical adaptations to drugs of abuse and alcohol are especially profound in midbrain dopaminergic neurons. Long-lasting molecular and structural changes in mesolimbic **dopaminergic** neurons that result from chronic exposure to drugs of abuse and alcohol are thought to underlie adverse behaviors such as compulsive drug seeking and relapse. Recent studies suggest that a subset of these changes is prevented¹ reversed by activation of the glial cell **line-** derived neurotrophic factor (GDNF) signaling pathway. Behavioral effects of drugs of abuse such as cocaine and alcohol are also negatively regulated by GDNF: inhibition of the endogenous GDNF pathway enhances the activity of drugs of abuse, while administration of GDNF reduces the severity of the effects. In this review, we summarize the data implicating GDNF as a negative regulator of drug and alcohol addiction. We also provide evidence to suggest that therapies that activate GDNF signaling may be useful for the treatment of drug and alcohol addiction.

KEY WORDS

GDNF, alcohol, alcohol self-administration, cocaine, dopamine, ibogaine, ventral tegmental area

INTRODUCTION

Glial cell line-derived neurotrophic factor (GDNF) belongs to the transforming growth factor β (TGF- β) superfamily that also includes **neurturin**, **artemin** and **persephin** /1/. GDNF derives its name **from** its discovery; the factor was originally isolated from a glioma cell line, and subsequently shown to promote survival of mesencephalic dopamine neurons in culture /47/. GDNF plays an essential role in the development of kidneys and peripheral neurons /58/, and has also been found to promote neuronal survival of mesencephalic dopaminergic (**DAergic**) neurons in the adult following injury 14,791. As such, its potential as a therapeutic agent for the treatment of pathologies such as Parkinson's disease has been widely explored 13, 20,21,24,45/. More recently, a role for GDNF in addiction has been suggested. Interestingly, GDNF acts to counteract both biochemical and behavioral adaptations produced by drugs of abuse. In this review, we first describe the characteristics of the GDNF signaling pathway, and then discuss the evidence that GDNF alters the biochemical and behavioral effects of addictive drugs and alcohol.

GDNF-MEDIATED SIGNALING

GDNF activity is mediated via the Ret and Glial cell line-derived neurotrophic factor $\alpha 1$ (GFR $\alpha 1$) receptors /17,43,80,81/. Ret is the product of the **c-ret** proto-oncogene, and is a receptor tyrosine kinase that is alternatively spliced to yield two isoforms that differ in nine amino acids in their C termini /84/. The Ret receptor is shared by all four members of the GDNF family of growth factors 115,811; specificity for GDNF is conferred by GFR $\alpha 1$, a **glycosyl-phosphatidyl inositol** (GPI)-linked co-receptor /17,43,80/. Activation of the GDNF pathway is initiated via the ligation of GDNF to GFR $\alpha 1$, and the recruitment of Ret to the

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GFR α 1-GDNF complex /43/. GFR α 1-Ret association can occur when both are expressed on the same cell (cis signaling), or when GFR α 1 is presented as a soluble form (trans signaling) 156, 80,911. Upon complex formation, Ret forms dimers and becomes phosphorylated at four tyrosine sites (Tyr905, Tyr1062, Tyr1015 and Tyr1096) via autophosphorylation /13,151. The phosphorylated tyrosines interact with distinct adaptor and signaling proteins that initiate activity of several different downstream signaling pathways, including the activation of the mitogen activated protein (MAP) kinases ERK1 and ERK2, as well as the phosphatidylinositol 3 kinase (PI3K), phospholipase C γ (PLC γ), and cyclin-dependent kinase 5 (CDK5) cascades /1/. Activation of these cascades has consequences for gene transcription, in that activation of the Ret receptor via GDNF leads to the phosphorylation of the transcription factor cAMP response element binding protein (CREB), and increased expression of the transcription factors NF κ B and c-fos /34,44,83/. Interestingly, GDNF has recently been shown to trigger signaling that is independent of Ret. For example, GDNF can bind to the neuronal cell adhesion molecule (NCAM), which subsequently stimulates cell migration and axonal outgrowth in hippocampal and cortical neurons /57/, and may participate in supporting DAergic neuronal survival and outgrowth 1121. Recently, GDNF has been found to promote cell migration of cortical γ -amino butyric acid (GABA)-ergic neurons in a process that requires GFR α 1, but that is independent of both Ret and NCAM 1621, suggesting the existence of more, as yet unidentified, GDNF receptors.

GDNF SIGNALING IS REGULATED BY RECEPTOR COMPARTMENTALIZATION IN LIPID RAFTS

An important factor in the regulation of the GDNF pathway is the compartmentalization of its receptors within the plasma membrane. The GPI-linked GFR α 1 is localized, at least in part, to lipid rafts /85/. Lipid rafts are membranous micro-assemblies of cholesterol and sphingolipids of the plasma membrane 1741. Lipid rafts are thought to function as platforms for signaling cascades, and, in neurons, lipid rafts are thought to have important

functions for growth factor signaling, cell adhesion, axon guidance and synaptic transmission 174,851. Recent studies suggest that lipid rafts play a role in Ret-mediated signaling. Upon GDNF binding to GFR α 1, the complex recruits Ret into the rafts, leading to the activation of Ret within the rafts 156, 781. Furthermore, several important mediators for Ret signaling, such as Src, are also components of the lipid rafts 1181, suggesting that lipid rafts are important microdomains for Ret activities. Interestingly, soluble GFR α 1 (trans activation) can also recruit Ret into the lipid rafts; however, the time course is much slower /56/, and Ret-mediated activation of signal transduction cascades within the lipid rafts is different from Ret-mediated raft-independent signaling 1781.

EXPRESSION AND DISTRIBUTION OF GDNF AND ITS RECEPTORS IN THE NERVOUS SYSTEM

In situ hybridization studies show that in the normal adult brain, the mRNA levels of GDNF are relatively low and are detected in only restricted brain regions 129,821. GDNF mRNA is found in areas such as the ventral and dorsal striatum, thalamus, olfactory bulb and cingulate cortex /82/. Interestingly, the expression of the GDNF receptors, GFR α 1 and Ret, does not consistently overlap that of GDNF itself. For example, the caudate-putamen and the accumbens express moderate levels of GDNF, but not GFR α 1 and Ret /54, 66,821. Both Ret and GFR α 1 are highly expressed in the midbrain /29,59,66,82/, but, under normal conditions, GDNF was detected by in situ hybridization in one study /59/, but not in others 129,821. Finally, some regions, such as the subthalamic nucleus, express high levels of Ret but not GDNF or GFR α 1, while other regions, such as the neocortex, express high levels of GFR α 1 but not Ret 182,911. The lack of a complete overlap between the trophic factor and its receptors in some regions suggests the existence of GDNF pathways which are independent of activation of these receptors, as indicated above, and that the expression of both the growth factor and its receptors can be regulated. Indeed, kainic acid treatment was found to induce GDNF and GFR α 1 expression in the hippocampus 140,821, and in *vivo* administration

of agents such as phencyclidine (PCP) and ibogaine have been shown to induce the expression of GDNF in the midbrain 135,691. Finally, treatment of C6 glioblastoma cells with fibroblast growth factor-2 (FGF-2) or antidepressant drugs also results in the induction of GDNF expression 137,751.

GDNF FUNCTIONS

Activation of the GDNF signaling pathways regulates various cellular functions (for review, see /1/). For example, GDNF-mediated signaling promotes cell differentiation 150,621, migration, and chemotaxis /76/, as well as midbrain DAergic neuron sprouting and survival /4,47,79/. Proper activation of the GDNF signaling pathway is essential for the embryonic development of the autonomic and enteric nervous systems and for kidney development /19,53,58,65,67/; consequently, GDNF-deleted mice die shortly after birth /53,58, 65/. Mutations in the *Ret* gene which result in the inactivation of the receptor kinase cause Hirschsprung's disease 1681, and *Ret* mutations that result in constitutively-active *Ret* kinase lead to multiple endocrine neoplasia (MEN) syndromes 2A and 2B 142,601. More recent evidence suggests that GDNF contributes to synaptic transmission /9,86,90/, as well as to learning and memory in the adult; mice with a deletion of one copy of the GDNF gene are impaired in acquisition of a spatial memory task /25/.

In summary, GDNF plays an important role during development and also in adulthood. It is possible that one or more of these cellular functions of GDNF contribute to the inhibition of drug and alcohol effects by GDNF. This inhibitory effect and its possible mechanism(s) are discussed below.

GDNF AND ADDICTION

As described above, the GDNF receptors, GFR α 1 and *Ret*, are highly expressed in the ventral tegmental (VTA) midbrain DAergic neurons 129, 66,821, and GDNF affects DA neuronal function /4, 791. It is therefore likely that GDNF influences many of the behaviors regulated by midbrain DAergic cell groups. Midbrain DAergic neurons

within the VTA are a critical component of the neural circuitry involved in drug- and alcohol-seeking behavior. This conclusion derives from the finding that all drugs of abuse enhance extracellular dopamine (DA) levels within VTA terminal regions, and that DA antagonists injected into these same VTA terminal regions potentially alter drug and alcohol self-administration and relapse /14,41,46, 72,881.

VTA DAergic neurons are also selectively vulnerable to some neuroadaptations induced by repeated exposure to drugs of abuse. For example, chronic exposure of rodents to morphine, cocaine or alcohol, as well as cocaine self-administration, increases levels of the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) glutamate receptor subunit GluR1, and the NR1 subunit of the *N*-methyl-D-aspartate (NMDA) glutamate receptor /22,48,55,77/. Drug exposure also leads to alterations in levels of signaling proteins within the VTA. For example, chronic morphine is reported to increase the levels of PLC γ 1 /89/, decrease the expression of α -synuclein /92/, and chronic cocaine and morphine treatment both enhance levels of phosphorylated ERK1 and ERK2 (extracellular signal-regulated kinase) in the VTA, but not in the neighboring DAergic cells of the substantia nigra (SN) /6/. There is evidence that the drug-induced changes in ERK1 activity lead to alteration in the level of tyrosine hydroxylase (TH) /7,8,55/, the rate-limiting enzyme in the biosynthesis of catecholamines, including DA 1301. In addition, VTA adaptations to chronic exposure to cocaine and morphine are associated with alterations in nucleus accumbens signaling pathways: increases in levels of the immediate early gene, Δ FosB, and of the PKA catalytic subunit (PKA Ca) are detected upon exposure of rodents to chronic cocaine and morphine 138,511. It is possible that some of the VTA responses to drugs of abuse reflect a response to the drug as an external toxin, as levels of glial fibrillary acidic protein (GFAP) are enhanced following chronic morphine, cocaine, and alcohol exposure 17,331. Further evidence of insult following each of these chronic drug treatments can be seen in the reduction in neurofilament proteins, again, an effect that is selective for the VTA, but not SN, DAergic neurons 15,551. Taken together,

these findings indicate that there are common molecular changes produced by repeated administration of at least some of the major drugs of abuse, although, not surprisingly, the time-course of the appearance and disappearance of these changes is dependent on the specific treatment parameters. Interestingly, drug-induced changes in the GDNF system have also been reported. Nestler and colleagues found that chronic cocaine and morphine exposure decreases the levels of phosphorylation (and thus activation) of the Ret receptor in the VTA /51/. Hence, drug treatments that produce cellular and molecular changes in the VTA also negatively alter the signaling pathway for the growth factor, GDNF. Additionally, drug-induced changes in the GDNF system in brain regions other than the VTA have also been reported. For example, systemic administration of phencyclidine (PCP) for 5 days was found to increase expression of GDNF in the VTA and the SN /69/, while Green-Sadan *et al.* reported that GDNF mRNA levels were reduced in the dorsal but not ventral striatum after 12 days of cocaine self-administration /31/.

Because GDNF promotes the survival and growth of DAergic neurons after injury (see above), it is possible that the changes in the VTA that accompany repeated drug and alcohol use result from downregulation of the GDNF system that would normally serve to counter these changes. If this is true, then GDNF should prevent these biochemical changes. In an elegant study, Messer *et al.* /51/ found that infusion of GDNF into the VTA blocks and/or reverses cocaine-induced increases in the NR1 subunit of the NMDA receptor, as well as the nucleus accumbens changes in Δ FosB and PKA α 1511. GDNF is known to regulate the level of TH in the midbrain /21,23,64/, and specifically in the VTA /52/; Messer *et al.* found that intra-VTA GDNF also reverses cocaine-induced increases in VTA TH /51/. The behavioral relevance of this intra-VTA GDNF treatment that blocks the biochemical effects of cocaine was demonstrated: the same treatment also blocked the behavioral effects of repeated exposure to cocaine measured by the conditioned place preference procedure /51/. These findings suggest that prevention of neuroadaptations in the VTA by GDNF can block the develop-

ment of some behavioral features of addiction.

Since exogenous administration of GDNF blocks the effects of repeated administration of drugs of abuse, it is possible that endogenous GDNF systems may serve to counter the effects of these drugs, in line with its role as a neuroprotective agent. Messer *et al.* /51/ found evidence that endogenous GDNF signaling contributes to the effects of repeated cocaine in that intra-VTA infusion of anti-GDNF inhibitory antibodies, which prevent GDNF from activating its receptors, enhances the biochemical and behavioral adaptations to cocaine. Furthermore, GDNF heterozygote knockout mice are more sensitive to the rewarding and sensitizing effects of cocaine /51/. Together, these studies provide strong evidence that GDNF in the VTA serves to inhibit biochemical and behavioral adaptations to cocaine.

Our recent work supports a role for GDNF in the VTA in countering the addictive effects of alcohol. We found that GDNF reverses chronic alcohol induction of TH protein levels in a cell culture model (unpublished results). In addition, GDNF levels control alcohol intake, in that intra-VTA injection of GDNF reduces operant self-administration of alcohol /35/. Thus, the combined evidence suggests that GDNF in the VTA can counter biochemical and behavioral effects of cocaine and alcohol. However, it is important to note that the anti-addiction effects of GDNF may not be limited to the VTA. Recently, Green-Sadan *et al.* /31,32/ reported that transplantation of simian virus-40 glial cells that produce and secrete GDNF, or delivery of GDNF-conjugated nano-particles, into the dorsal and ventral striatum, impaired the acquisition of cocaine self-administration.

GDNF MEDIATES THE DESIRABLE ACTIVITIES OF THE ANTI-ADDICTION DRUG, IBOGAINE

If GDNF counteracts biochemical and behavioral neuroadaptation to drugs of abuse, then it could potentially be a useful drug target for anti-addiction medication development. Support for this possibility stems from a recent study in which we found that the anti-addiction drug ibogaine reduces alcohol consumption via activation of the GDNF pathway /35/.

For the past 30 years, ibogaine, a natural alkaloid found in the root bark of the African shrub *Tabernanthe iboga*, has attracted interest for its purported efficacy in reversing addiction to multiple drugs of abuse including alcohol [27,49,61]. Anecdotal reports of effects in humans indicate that ibogaine reduces craving and withdrawal symptoms [12,73]. Animal studies also suggest that ibogaine attenuates drug- and alcohol-induced behaviors. For example, ibogaine reduces heroin operant self-administration [16], and *naloxone*-precipitated withdrawal in morphine-dependent rats [11,28]. Ibogaine also decreases cocaine-induced locomotor activity and self-administration in rats [10] and mice [17,71]. Similar results have been reported for ibogaine's activities on alcohol intake in a two-bottle choice paradigm [13,63]. We showed that systemic administration of ibogaine reduces operant self-administration of alcohol in rats [35], as well as the binge drinking that occurs after a period of abstinence, demonstrating ibogaine's efficacy in a relapse model [13]. Importantly, we found that the VTA is a site of action for ibogaine's reduction of alcohol self-administration, and that a concentration of ibogaine that decreases alcohol consumption also increases GDNF expression in the midbrain of rodents [35]. We therefore tested whether GDNF mediates the activities of ibogaine, first in cells in culture, and then in vivo. We used the DAergic-like SH-SY5Y cell line to demonstrate that ibogaine exposure results in increased expression of GDNF and increased secretion of the GDNF protein, as well as the activation of the GDNF signaling cascade via the activation of the Ret receptor [35]. Finally, we showed that delivery of inhibitory antibodies against GDNF into the VTA attenuated ibogaine's decrease of alcohol operant self-administration in rats [35]. Because it is known that GDNF can alter the VTA-dependent biochemical and behavioral effects of cocaine and morphine (see above), the evidence as a whole suggests that ibogaine reduces self-administration of multiple drugs of abuse via GDNF.

SUMMARY AND FUTURE DIRECTIONS

In summary, repeated administration of drugs of abuse and alcohol induces a common pattern of changes in gene expression and protein levels selectively in the VTA. A subset of these changes is reversed by intra-VTA GDNF, as are some of the drug-induced behavioral effects. Endogenous GDNF systems appear to inhibit drug-related behaviors, while repeated drug administration appears to inhibit GDNF signaling itself. Based on these studies, we propose that GDNF is an endogenous anti-addiction agent. This possibility is directly supported by the finding that the activity of the anti-addiction drug, ibogaine, on alcohol consumption is mediated via increased expression of GDNF in the midbrain and the subsequent activation of the GDNF pathway. The exact mechanism of action of GDNF to counter the biochemical and behavioral adaptations associated with addiction is currently unknown. Below, we discuss findings from studies of the effects of GDNF on DAergic cell function that provide interesting avenues to explore.

As reviewed above, the site of action for the anti-addiction effects of GDNF appears to be the VTA. The GDNF receptors, Ret and GFR α 1, are highly expressed in midbrain DAergic cells within the SN and the VTA of adult brain [26,29,66,82], indicating that GDNF likely contributes to the function of midbrain DAergic neurons in the adult. GDNF produces effects on DAergic systems within both a short time frame of minutes to hours, and a longer period of days or even weeks. Recent findings indicate that acute application of GDNF to midbrain DAergic neuronal cells in culture activates the MAP kinase pathway within 5-15 minutes, resulting in the inhibition of type-A potassium channels and enhanced neuronal excitability [19]. In addition, it appears that GDNF can potentiate Ca²⁺ channels [18]. These findings suggest that GDNF can have rapid effects on DA release, although this remains to be demonstrated. In contrast to its effects on ion channels, the majority of studies of GDNF have measured changes in DA systems over longer time spans. As might be expected from its enhancement of morphological features of DAergic neurons in cell culture, acute GDNF administration to the SN

increases DA neuronal sprouting /39/ and extracellular DA levels in the striatum of adult rats /36/. In addition, these findings have been extended to the neighboring DA cell group in the midbrain, the VTA. In cultures of VTA neurons, chronic treatment with GDNF is reported to increase the number of synapses onto DAergic, but not GABAergic neurons, and to enhance DA and glutamate release /9/, possibly via increased expression of the calcium binding protein, frequenin /86/. Finally, recent studies found that prolonged delivery of intra-striatal GDNF in *vivo* results in decreased expression and activity of TH 123,641, indicating that GDNF-mediated stimulation of DAergic neurons may activate a compensatory mechanism that leads to decreased levels of TH gene expression and activity. Taken together, these findings indicate that GDNF can alter the neuronal activity of DA neurons in the VTA through multiple mechanisms. It is likely that GDNF-mediated regulation of DA synthesis and release by VTA DA neurons, through a combination of short-term effects on ion-channels and longer-term effects on TH regulation and synaptic remodeling, underlies the ability of GDNF to counter the addictive effects of alcohol and other drugs of abuse.

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